# DIRECT PROTON COUPLED ELECTRON TRANSFER IN THE SYSTEM GOLD ELECTRODE - RECOMBINANT HORSERADISH PEROXIDASE

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Horseradish peroxidase (HRP), a hem-containing glycoprotein, is able to utilize hydrogen peroxide to catalyze one electron oxidation of a wide variety of organic and inorganic substrates. The direct electrochemical reduction of the oxidized form of HRP adsorbed at the electrode, kinetically slow on a majority of the investigated electrode materials with values of the electron transfer (ET) rate, generally, below 1 electron per second, may be referred to the type of ET reactions accompanied by proton transfer (PT) if ET between the surface of the electrode and the active center of the enzyme can be established [1].

To verify the reaction mechanism involving the participation of a proton in the elementary step of the charge transfer, the kinetics of the bioelectrocatalytic reduction of hydrogen peroxide has been studied on gold electrodes modified with different forms of HRP placed either in a rotating disk assembly or in a wall jet flow through electrochemical cell. Native HRP and non-glycosylated recombinant forms, rec-HRP and rec-HRP containing a six-histidine tag at the C-terminus, $C_{His}$rec-HRP, have been used for adsorptive modification of gold electrodes. A favorable adsorption of recombinant HRPs on gold from a protein solution in 0.01 M phosphate buffer at pH 6.0 containing 0.15 M NaCl (PBS) provided a high and stable current response to $H_2O_2$ due to its bioelectrocatalytic reduction based on direct (mediatorless) ET between gold and the active site of HRP [2]. The presence of the six-His tag at the C-terminus of the enzyme molecule additionally increased the strength of the binding of the enzyme with the gold surface as well as the efficiency of direct ET.

The heterogeneous ET rate constant, $k_s$, calculated from data from experiments on direct ET, additionally on mediated ET in the presence of catechol as well as from microbalance data, increased 30 times in the sequence native HRP - rec-HRP - $C_{His}$rec-HRP. Comparative estimations with the Marcus theory [3] with the values of the ET rate constants extrapolated to zero concentration of $H_3O^+$ (electronic coupling constant $\beta$ assumed to be 10 $nm^{-1}$) resulted in a decrease in the distance between the active site of the adsorbed enzyme and the electrode of 0.34 nm when comparing $C_{His}$rec-HRP with native HRP. The values of the apparent heterogeneous ET rate constant between $C_{His}$rec-HRP and gold changed from a value of 82.9±22.1 $s^{-1}$ in PBS at pH7.4, to a value of 288±68 $s^{-1}$ in PBS at pH 6.0, and similar data on the relative increase of $k_s$ were also obtained for the other HRP forms. These data support the assumption of a simultaneous electron and proton transfer as the limiting step of the charge transfer developed for the electroreduction of the oxidized form of native HRP on gold [1]. The results demonstrate new possibilities for controlling and enhancing the enzymatic reactivity and efficiency of coupled electron and proton transfer reactions at the electrode/solution interface.

REFERENCES

1. Ferapontova E. E., Gorton L. Effect of Proton Donors on Direct Electron Transfer in the System Gold Electrode - Horseradish Peroxidase: Simultaneous Electron and Proton Transfer. Bioelectrochemistry (2001) submitted.

2. Ferapontova E. E., Grigorenko V.G., Egorov A. M., Börchers T., Ruzgas T., Gorton L. Mediatorless Biosensor for $H_2O_2$ Based on Recombinant Forms of Horseradish Peroxidase Directly Adsorbed on Polycrystalline Gold. Biosens. Bioelectron. (2001) in press.

3. Marcus R.A., Sutin N. Electron Transfers in Chemistry and Biology. Biochim. Biophys. Acta, 811 (1985) 265-322.